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Membrane Fusion: The Role of Polyphosphatidylinositol

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We have found that the presence of the phospholipid, lysophosphatidylinositol monophosphate (LPIP) correlates positively with the fusion potential of fusogenic carrot cells. There was no correlation with the presence of phosphatidylinositol monophosphate (PIP) and phosphatidylinositol bisphosphate (PIP_2) and fusion. Nor was there evidence for the need for PIP or PIP_2 turnover in order for the cells to be fusion permissive. LPIP was synthesized primarily from the phosphorylation of lysophosphatidylinositol. Lysolipids were found to decrease the phosphorylation of PI and PIP suggesting a mechanism for regulating the biosynthesis of the polyphosphoinositides which are key components of the signal transduction pathway in many animal cells.

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MEMBRANE FUSION: THE ROLE OF POLYPHOSPHATIDYLINOSITOL

FINAL REPORT

Wendy F. Boss and Jeffery J. Wheeler

U. S. Army Research Office

Grant Number: DAAL03-86-G-0035

North Carolina State University

Brief Outline of Research Findings

The focus of this research was to investigate the role of the polyphosphoinositides in cell fusion. As a result of our research we have found that the presence of the phospholipid, lysophosphatidylinositol monophosphate (LPIP) correlates positively with the fusion potential of fusogenic carrot cells. There was no correlation with the presence of phosphatidylinositol monophosphate (PIP) and phosphatidylinositol bisphosphate (PIP_2) and fusion. Nor was there evidence for the need for PIP or PIP_2 turnover in order for the cells to be fusion permissive.

Although LPIP was a known lysophospholipid, it had not been reported in a living system prior to our work. For this reason we were careful to identify the lipid and have used fast atom bombardment mass spectrometry to identify the fatty acids associated with the lipid.

We also found that LPIP was synthesized by ATP-dependent phosphorylation of LPI. While LPI was found primarily in the intracellular membranes LPIP was found predominantly in the plasma membrane. Kinase activity, however, was found in both membrane fractions in the in vitro assay system. Thus the question remained as to what regulated the biogenesis of the plasma membrane LPIP in vivo. This has been addressed to some extent in a paper recently submitted for publication. Conditions which favor formation of LPI from phospholipase A_2 hydrolysis of PI (acidic pH, pH 5.5) do not favor kinase activity. Thus our working hypothesis is that the LPI is synthesized on the intracellular membranes in an acidic secretory vesicle and transported to the plasma membrane where it is phosphorylated.

In addition, we found that the lysolipids affect the biosynthesis of PIP and PIP_2 which are involved in signal transduction in many animal cells. Thus the lysolipid pathway may be important for regulating cellular signalling as well as membrane fusion.

We were unable to do biophysical studies using spin labeled polyphosphoinositides due to time constraints and the difficulty in synthesizing derivatives of these lipids. To my knowledge there are no published reports of spin label phosphoinositides, although Dr. S. McLaughlin says his lab has recently been successful in synthesizing a fluorescent analogue.

During the three years of the fellowship, Dr. Wheeler has had two manuscripts published in journals, co-authored two book chapters (first author of one) and has two manuscripts submitted for publication. These are listed below and a copy of the latest manuscripts is attached.

We feel very fortunate to have had support from the Army Research

Office to pursue this work. While we have gained new information about the structure of a naturally fusing cell membrane and the biosynthesis of lysophosphoinositides, we have produced a fine scientist who has had offers for postdoc postions from excellent laboratories. Dr. Wheeler will be joining either the laboratory of Dr. Pieter Cullis or Dr. R. M. Epand to pursue biophysical studies of fusion. Thank you for your support.

Publications:

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Degrees awarded: Ph. D. Jeffery J. Wheeler. May, 1989

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